

**Pre-BTAG Meeting for the Libby OU3 Site
October 30-31, 2007**

Meeting Attendees:

Bonita Lavelle, USEPA, Region 8 – Libby OU3 RPM
David Charters, USEPA, Edison, NJ
Rich Henry, USFWS, Edison, NJ
Karen Nelson, USFWS Helena Montana
Dan Wall, USFWS liaison to USEPA, Region 8
Janet Burris, Syracuse Research Corporation (SRC) (EPA Contractor)
Lynn Woodbury, Syracuse Research Corporation (SRC) (EPA Contractor)

The meeting began with introductions and a review of asbestos analytical methods by Mary Goldade from USEPA Region 8.

Review of Asbestos Analytical Methods

Mary provided an overview of the available analytical methods for asbestos analysis in air, water, soil/sediment and tissues. Asbestos measurement techniques were discussed by the following methods:

Phase contrast microscopy (PCM) - Light is transmitted through a sample and in order to visualize its components. Structures are counted at low resolution (5 to 200X) but fibers cannot be distinguished (as asbestos or another type of fiber).

Polarized light microscopy (PLM) - Light is transmitted through a sample and then filtered with a polarizing lens in order to visualize its components. It is possible to identify asbestos morphology and mineral type. Quantity is estimated by Visual Area estimation by counting structures and estimating weight as %. Structures are counted at low magnification (5 to 400X).

Electron Microscopy - Electrons are used instead of light to visualize the specimen. Instead of glass lenses focusing the light wavelengths, electromagnetic lenses are used to focus the electrons on the sample.

Transmission Electron Microscopy (TEM) – It is possible to assess Asbestos morphology (2 dimensional), Asbestos mineral & crystalline structure and count structures at higher magnification (≈500 – 20,000X).

Scanning Electron Microscopy (SEM) - It is possible to assess Asbestos morphology (3 dimensional), mineral type and count structures at high magnification (≈50 – 10,000X).

Asbestos is divided into two mineral groups: serpentine and amphibole. Chrysotile is the only type of serpentine asbestos, possessing relatively long, thin and flexible crystalline fibers. Amphibole asbestos are shorter, needle-like and substantially more brittle than serpentine asbestos. The type of asbestos at Libby is amphibole. EDS (Energy Dispersive Spectroscopy) is used to determine the mineralogy of a structure. SAED (Selected Area Electron Diffraction) is used to determine information on crystalline structure to aid in confirmation of material. Mary then described the different counting rules used in asbestos analyses. Counting rules and procedures vary with the methodology. The aspect ratio is equal to length divided by width. In PCM fibers greater than 5 μm in length are counted with an aspect ratio of 3:1. In TEM lengths fibers greater than 0.5 μm are counted with an aspect ratio of 3:1 or 5:1 depending on methodology used. For Libby OU3 all TEM samples all fibers greater than 0.5 μm with an aspect ratio of 3:1 are counted.

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Sensitivity of asbestos analyses is a function of the amount of the sample examined where $S(fx)$ = amount of sample/portion of sample examined. The sensitivity of asbestos analyses can be increased by increasing the surface area analyzed (number of grid openings (GOs) examined). But this comes with a cost. Mary provided the following example:

Volume of Air (Liters)	#GO	Sensitivity (S/cc)	Approx. Cost
2,500	10	0.0012	~\$80
2,500	30	0.0004	~\$280
2,500	50	0.0002	~\$480

Sample preparation may also influence sensitivity. Sample preparation influences sample representativeness and analytical sensitivity. The principles are to maximize the sample size and concentrate Libby Amphibole while reducing sample matrix. Mary reviewed each environmental matrix (air, water, soil/sediment and tissue) with regard to sample types, the requirements for sample separation and preparation, analytical methods, reporting units, and the quantitative vs qualitative nature of the results. The following matrix table provides a summary of the

Analyses of Asbestos in Air

- Sample types – Air samples are collected as stationary samples or personal activity based samples.
- Separation/Preparation – Not usually required unless there is overloading of the filters.
- Analytical Method – TEM
- Units of Measure – LA structures per cubic centimeter of air
- Quantitative data

Analyses of Asbestos in Water

- Sample types – Surface water and groundwater
- Separation -- Yes, water filtered out leaving only particulates (asbestos + other material)
- Preparation -- Organic reduction (e.g., ashing) and Inorganic reduction (e.g., chemicals)
- Analytical Method -- TEM for bivariate distribution
- Units of Measure
The typical unit is number of fibers $>10\mu\text{m}$ per liter of water as these are the units for the drinking water standard for asbestos. For OU3, all fibers are counted with an aspect ratio of 3:1 regardless of length.
- Quantitative data

Analyses of Asbestos in Soil/Sediment

- Preparation/Separation Options --Dry and grind to homogenize, sieve in series, liquid, (e.g., sedimentation or centrifugation), and “pick and place”
- Analytical Methods -- PLM-VE, PLM-PC, PLM-grav, TEM or SEM
- Units of Measure-- PLM-VE: % LA (by weight); TEM: LA structures per gram of soil or sediment

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- Quantitative or Qualitative Data? --PLM-VE: Semi-Quantitative (0.2% LA); PLM-grav: Quantitative; PLM-PC: "Quantitative"; TEM: Qualitative, Semi-Quantitative? (presence/absence)
 - TEM produces results in units of LA Structures per gram, but results are not quantitatively reproducible
 - Beyond PLM-VE, PLM-PC, PLM-grav, soil or sediment analyses will require method development and validation for quantitative results

Analyses of Asbestos in Tissues

- Fixation Required - Formalin vs freeze drying
- Separation Required - Yes, remove organic material (digestion vs ashing)
- Analytical Method - TEM or SEM for bivariate distribution
- Units of Measure - LA Structures per gram of tissue (dry wt)
- Quantitative or Qualitative Data? -Depends on level of method validation
Qualitative, Semi-Quant, Quantitative

There was some confusion over the term "fixation". Individuals present who were familiar with histological examination of tissues use the term "fixation" for preparation of tissue samples for histological examination where Mary was using the term to describe preservation of the tissues. Two action items were identified at the close of the discussion of asbestos analytical methods including:

- SRC will distribute copies of the TEM training notes from the November 2006 database training sessions, as well as a copy of the ISO 10312 method.
- SRC will prepare a matrix which summarizes the differences between and advantages/limitations of each analytical method.

Review of Existing Data for OU3

Dan Wall, the United States Fish and Wildlife (USFWS) liaison to USEPA Region 8 provided a brief summary of the mine history and presented the limited datasets for environmental media that were available for OU3 prior to the current Phase 1 investigation. Dan also presented photographs of Rainy Creek, the tailings impoundment, and mill pond from a site visit in September 2007. There was some discussion concerning the accuracy of the estimated mass releases of asbestos from the mine operations. Tailings were deposited directly to Rainy Creek prior to the installation of the tailings impoundment. Dan stated that habitat classification had been completed for the area and there is some Rainy Creek data. Two action items were identified at the close of the discussion of existing data including:

- SRC will refine the OU3 site boundary map presented in the Phase I SAP (Figure 2-1) to more clearly identify the inclusion/exclusion of parcels near the boundary and ensure that the boundary encompasses the Kootenai River.
- SRC will investigate a potential typo in the surface water summary table (number of LA structures > 10 um for sample 1R-06027).

Conceptual Approach for the Ecological Risk Assessment

Possible approaches to the ecological risk assessment were discussed for asbestos contamination by receptor group (mammals, birds, aquatic organisms, terrestrial invertebrates, amphibians, and terrestrial plants) using conceptual diagrams for each that described possible steps and methods for establishing nature and extent, screening level assessment and the baseline risk assessment. For non-asbestos

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contaminants the ecological risk assessment will include a screening level assessment in which chemical concentrations in environmental media will be compared to applicable screening benchmarks. The baseline ecological risk assessment will then focus only on those chemicals of potential concern as identified from the screening level assessment. There were not parallel discussions concerning the non-asbestos methodology as these are fairly well established.

Mammals

For mammals, the discussion began with Janet providing a review of the available toxicity literature for asbestos and mammals. A literature search has been completed and some toxicity literature is available for mammals exposed to asbestos via inhalation, injection (intratracheal, etc.,) and ingestion (drinking water, diet, and gavage) exposures. Members of the group agreed that injection exposures were not appropriate for use. Most studies are based on exposures to chrysotile but some are based for amosite, crocidolite, and taconite exposures. No studies are available for exposures to Libby amphibole (LA) asbestos. Members of the group agreed that toxicity data for other types of asbestos (especially amosite) could be used to derive toxicity reference values with the uncertainties discussed. Exposure units are reported in the literature in multiple forms including mass per org per day, fibers per liter, % of diet, fibers per cc. Conversion to dose unit will require some level of effort and assumptions. Endpoints that are reported include effects on reproduction, growth, survival, pathology, physiology, bioaccumulation and biochemical.

Because of several limitations (including the inability to measure asbestos concentrations in soil quantitatively, limitations in the toxicity data and the difficulty in estimating inhalation exposures (we do not have an analogous method for activity based sampling at this time for mammalian receptors)) and the known toxicity of asbestos, the group decided that the "typical" screening assessment could not be performed. By "typical" the group was referring to the comparison of a screening level (media based concentration) considered safe to measured site concentrations (in soil and air). The available toxicity literature does establish that a hazard potential exists; therefore, the ecological risk assessment for asbestos will bypass an initial screening assessment (to determine if asbestos warrants proceeding to a full baseline assessment) and proceed directly to a baseline risk assessment.

The discussion of the potential scope of the baseline risk assessment centered on the conceptual diagrams provided. Sampling of small mammal tissues for asbestos burden (target tissues such as lung and gastrointestinal tract) could be sampled along a contamination gradient to identify if exposures are occurring to small mammals. Further examination of the histology of the target tissues would identify the level and type of abnormalities in asbestos exposed animals in comparison to a reference. Collection of community metrics could provide a "snapshot" of the diversity and density of mammals on the mine site in comparison to a reference. Some follow up questions concerned:

- What will be the spatial extent sampled? Radiating transects with ¼ mile sampling locations (similar to the forest soil/tree bark sampling)? Close, near, far, off-site reference areas?
- What are the species of interest?
- What characteristics will the off-site reference area have (e.g., disturbed vs. undisturbed)? One off-site reference area that has been proposed is near a disturbed gravel pit.

Some action items that were identified included:

- SRC will develop a matrix that summarizes the pros and cons of several potential representative species of interest.
- SRC will obtain USGS Quadrant maps for mine area and potential reference areas of interest.

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- SRC will identify with the assistance of EPA field collection experts to provide input on the appropriate methods and equipment necessary for small mammal collection.

Birds

A discussion parallel to that of mammals ensued concerning avian receptors. The same approach would apply for birds including the collection of tissues for asbestos content, histopathology evaluation, and community metrics. However, some concerns were expressed that the collection of community metrics for birds may not be feasible and that the interpretation of histopathology may to be possible. There has only been one study identified for birds exposed to asbestos (a single study with chickens exposed via intratracheal injection). In addition the follow-on questions identified for mammals some additional follow-on questions were identified for birds:

- What are the target tissues for tissue burden and histopathology evaluations?
- Is it feasible to perform a laboratory dosing study to relate tissue burdens to effects endpoints and assess potential mechanisms of action? To identify an asbestos (LA specific) dose response value for birds?

Several action items were identified from the discussion including:

- SRC will identify possible experts in the fields of avian pathology, physiology and/or toxicology that will be able to provide guidance on how asbestos may affect the respiratory function of birds. These experts may also be to identify potential target tissues and biomarkers of effect from asbestos exposures. Two individuals that were identified during the meeting were Crystal Driver and Anne Fairbrother.
- Karen Nelson volunteered to investigate potential permitting requirements associated with the Migratory Bird Act that need to be met for collection of birds from the site and reference areas.
- As needed, SRC will identify field collection experts to provide input on the appropriate methods and equipment necessary for bird collection.

Aquatic Invertebrates

Primary focus for aquatic invertebrate sampling efforts will be Rainy Creek, the tailings impoundment pond, and the mill pond, as well as a reference stream and ponded area. The reference stream and ponded area were selected based on the site visit by Dan Wall and Karen Nelson in September 2007. As data become available, exposure and toxicity information from Rainy Creek will be used to guide potential sampling efforts in the Kootenai River. The ensuing discussions did not consider the Kootenai River.

Toxicity data for asbestos are available for a only limited number of aquatic invertebrates. Toxicity data are available for chrysotile asbestos to several fish species. It would be possible to establish a screening benchmark for surface water but it would be for chrysotile and not LA. The available toxicity literature does establish that a hazard potential exists; therefore, the ecological risk assessment for asbestos will bypass an initial screening assessment (to determine if asbestos warrants proceeding to a full baseline assessment) and proceed directly to a baseline risk assessment. The possible approach for asbestos for the baseline risk assessment for aquatic invertebrates will be similar to that for non-asbestos with the exception that toxicity benchmarks for sediments for asbestos are not available. The following possible components of the baseline were discussed:

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- Standard toxicity tests with standard test organisms exposed to site media (surface water and/or sediment)
- Toxicity identification evaluation (TIE) tests to rule out toxicity from non-asbestos parameters
- Spiking studies for sediment to quantify asbestos levels associated with adverse effects and if adverse effects are observed below analytical detection limits for PLM-VE
- Collection of community metrics to provide a “snapshot” of diversity/density in the streams (not the ponds)

There was some discussion concerning the usefulness of a sediment toxicity test with either site collected sediment samples as it is not possible to quantify the amount of asbestos in sediment. This issue was not resolved and the parties moved on to a discussion of possible approaches for fish. The following follow-on questions were identified for the baseline risk assessment for aquatic invertebrates:

- What is the appropriate exposure media (water, sediment) and route of interest?
- What media and test organism(s) should be used in the toxicity tests?
- What endpoints will be assessed as biomarkers of asbestos exposure and effect?

The following possible components of the baseline risk assessment for fish exposed to asbestos in water and sediment:

- Standard toxicity tests for trout species with exposure to site surface water
- Spiking studies for surface water to guide histopathology evaluation with regard to target tissues and biomarkers of effect and to identify dose-response values for LA
- Tissue burden of asbestos in target tissues of fish
- Histopathology evaluation (gill, lateral line, kidney) from field collected fish
- Collection of community metrics to provide a “snapshot” of diversity/density in the streams

The primary focus for fish sampling efforts will be Rainy Creek and a reference stream. The reference stream was selected based on a site visit by Dan Wall and Karen Nelson in September 2007.

The following follow-on questions were identified for the baseline risk assessment for fish:

- Which trout species should be selected for use in the toxicity tests?
- Are both spiking studies and exposures with field collected samples necessary?
- What types of adverse histopathology (and in what target organs) are associated with asbestos exposure in fish?

Terrestrial Plants

The primary focus for the baseline risk assessment for terrestrial plants will focus on asbestos contaminated vegetated areas adjacent to the disturbed mine area. An evaluation of “on-site” areas (e.g., the disturbed mine area) will be deferred to the Feasibility Study, at which time re-vegetation evaluations will consider chemical (organic content, macronutrients), physical (moisture content), and asbestos-related impacts to plants. The group agreed that the text discussion of the site conceptual model [in the baseline ecological risk assessment] will include a brief overview of why organisms, such as terrestrial invertebrates [and plants], are important biological indicators of impacts and how these organisms are linked to other receptors of interest via the food chain.

The following possible components of the baseline risk assessment for terrestrial plants exposed to asbestos in soil:

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- Standard toxicity tests with exposure of standard test plants to site soils
- Spiking studies for soil to quantify asbestos levels associated with adverse effects and iff effects are observed below analytical detection limits for PLM-VE

The following follow-on questions were identified for the baseline risk assessment for terrestrial plants:

- Which plant species will be selected for use in the toxicity tests?
- What endpoints will be assessed as biomarkers of asbestos exposure and effect?

Terrestrial Invertebrates

The possible scope of the baseline risk assessment for terrestrial invertebrates was inferred from that on terrestrial plants.

Amphibians

Currently there are not toxicity studies available for amphibians exposed to asbestos. There are concerns about a protected species (the Boreal toad) that resides on the site. The following possible components of the baseline risk assessment for amphibians exposed to asbestos in water, soil and sediment include:

- Toxicity tests with exposure to early life stages
- Population and/or community surveys on site in comparison to a reference
- Examination of abnormalities in on site populations compared to a reference
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The following follow-on questions/action items were identified for the baseline risk assessment for amphibians:

- Karen Nelson will contact David Green (National Wildlife Health Center) for relevant standard operating procedures (SOPs) for amphibian population evaluations and abnormality assessments, and will provide this information to SRC.
- SRC will review SOPs for performing toxicity tests (e.g., FETAX), collecting population metrics, and performing abnormality assessments for amphibians to determine if these data will be useful in establishing/addressing data quality objectives.

Upcoming Schedule

Avian Meeting – The group members discussed a convening a meeting with avian experts to gather information on avian respiratory function, potential target tissues and biomarkers of effect from asbestos exposures.

BTAG Meeting 1 – The first Biological Technical Assistance Group (BTAG) meeting would present and discuss draft ideas for 2008 sampling in support of the ecological risk assessment. Included in this presentation would be the preliminary Phase I results from the fall 2007 sampling. Also included would be a draft Problem Formulation.

BTAG Meeting 2 – The second BTAG meeting would present and discuss the draft Phase II OU3 Sampling and Analysis Plan (SAP).

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All meetings will be open to all BTAG members. At this time it is anticipated that BTAG members will include representatives from USEPA, W.R. Grace, US Forest Service, USFWS, Montana Department of Environmental Quality (including the Department of Fish, Wildlife and Parks), the Libby TAG, and any state trustees.

Anticipated release of the Phase II OU3 is early-mid February 2008, with collection efforts to be conducted from Spring to Fall 2008. Bonnie emphasized that data collection activities may only be limited to a one year time span.